

AMENDMENTS TO THE SPECIFICATION.

After the abstract, please delete the existing sequence listing and insert the accompany sequence listing (pages 1-93).

At page 9, amend paragraph 0028 as follows:

[0028] In this regard, the first general step of linker design involves identification of plausible sites to be linked. Appropriate linkage sites on each of the V_H and V_L polypeptide domains include those which will result in the minimum loss of residues from the polypeptide domains, and which will necessitate a linker comprising a minimum number of residues consistent with the need for molecule stability. A pair of sites defines a "gap" to be linked. Linkers connecting the C-terminus of one domain to the N-terminus of the next generally comprise hydrophilic amino acids which assume an unstructured configuration in physiological solutions and preferably are free of residues having large side groups which might interfere with proper folding of the V_H and V_L chains. Thus, suitable linkers under the invention generally comprise polypeptide chains of alternating sets of glycine and serine residues, and may include glutamic acid and lysine residues inserted to enhance solubility. One particular linker under the invention has the amino acid sequence [(Gly)₄Ser]₃ (SEQ ID NO:1). Another particularly preferred linker has the amino acid sequence comprising 2 or 3 repeats of [(Ser)₄Gly] (SEQ ID NO:2) such as [(Ser)₄Gly]₃ (SEQ ID NO:3). Nucleotide sequences encoding such linker moieties can be readily provided using various oligonucleotide synthesis techniques known in the art. *See, e.g., Sambrook, supra.*

At pages 44-45, amend paragraph 0162 as follows:

[0162] To construct the vector pSYN3, a 1.5 kb stuffer fragment was amplified from pCANTAB5E (Pharmacia Biotech, Milwaukee, WI.) using PCR with the primers LMB3 (Marks, *et al.* (1991) *Eur. J. Immunol.* 21:985-991) and E-tagback (5'-ACC ACC GAA TTC TTA TTA ATG GTG ATG ATG GTG GAT GAC CAG CCG GTT CCA GCG G-3', (SEQ ID NO:1) (SEQ ID NO:4)). The DNA fragment was digested with *SfiI* and *NotI*, gel purified, and ligated into pCANTAB5E digested with *SfiI* and *NotI*. Ligated DNA was used to transform *Escherichia coli* TGl (Gibson (1991) *Studies on the Epstein-Barr virus genome. University of Cambridge, Cambridge, U. K.*), and clones containing the correct insert were identified by DNA sequencing.

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The resulting vector permits subcloning of phage-displayed scFv as *SfiI-NotI* or *Mcol-NotI* fragments for secretion into the periplasm of *E. coli* as native scFv with a C-terminal E epitope tag followed by a hexahistidine tag.

At pages 46-47, amend Table 1 as follows:

Table 1. Oligonucleotide primers used for PCR of mouse immunoglobulin genes.

Primer ID	Sequence	Seq I.D. No.
A. 1st strand cDNA synthesis		
Mouse heavy chain constant region primers		
MIgG1/2 For	5' CTG GAC AGG GAT CCA GAG TTC CA 3'	1 <u>5</u>
MIgG3 For	5' CTG GAC AGG GCT CCA TAG TTC CA 3'	2 <u>6</u>
Mouse □ constant region primer		
MC _K For	5' CTC ATT CCT GTT GAA GCT CTT GAC 3'	3 <u>7</u>
B. Primary PCR		
Mouse V _H back primers		
VH1 Back	5' GAG GTG CAG CTT CAG GAG TCA GG 3'	4 <u>8</u>
VH2 Back	5' GAT GTG CAG CTT CAG GAG TCR GG 3'	5 <u>9</u>
VH3 Back	5' CAG GTG CAG CTG AAG SAG TCA GG 3'	6 <u>10</u>
VH4/6 Back	5' GAG GTY CAG CTG CAR CAR TCT GG 3'	7 <u>11</u>
VH5/9 Back	5' CAG GTY CAR CTG CAG CAG YCT GG 3'	8 <u>12</u>
VH7 Back	5' GAR GTG AAG CTG GTG GAR TCT GG 3'	9 <u>13</u>
VH8 Back	5' GAG GTT CAG CTT CAG CAG TCT GG 3'	10 <u>14</u>
VH10 Back	5' GAA GTG CAG CTG KTG GAG WCT GG 3'	11 <u>15</u>
VH11 Back	5' CAG ATC CAG TTG CTG CAG TCT GG 3'	12 <u>16</u>
Mouse V _H back primers		
VH1 Back	5' GAC ATT GTG ATG WCA CAG TCT CC 3'	13 <u>17</u>
VH2 Back	5' GAT GTT KTG ATG ACC CAA ACT CC 3'	14 <u>18</u>
VH3 Back	5' GAT ATT GTG ATR ACB CAG GCW GC 3'	15 <u>19</u>
VH4 Back	5' GAC ATT GTG CTG ACM CAR TCT CC 3'	16 <u>20</u>
VH5 Back	5' SAA AWT GTK CTC ACC CAG TCT CC 3'	17 <u>21</u>
VH6 Back	5' GAY ATY VWG ATG ACM CAG WCT CC 3'	18 <u>22</u>
VH7 Back	5' CAA ATT GTT CTC ACC CAG TCT CC 3'	19 <u>23</u>
VH8 Back	5' TCA TTA TTG CAG GTG CTT GTG GG 3'	20 <u>24</u>
Mouse J _H forward primers		



JH1 For	5' TGA GGA GAC GGT GAC CGT GGT CCC 3'	21	<u>25</u>
JH2 For	5' TGA GGA GAC TGT GAG AGT GGT GCC 3'	22	<u>26</u>
JH3 For	5' TGC AGA GAC AGT GAC CAG AGT CCC 3'	23	<u>27</u>
JH4 For	5' TGA GGA GAC GGT GAC TGA GGT TCC 3'	24	<u>28</u>

Mouse JK forward primers:

JK1 For	5' TTT GAT TTC CAG CTT GGT GCC TCC 3'	25	<u>29</u>
JK2 For	5' TTT TAT TTC CAG CTT GGT CCC CCC 3'	26	<u>30</u>
JK3 For	5' TTT TAT TTC CAG TCT GGT CCC ATC 3'	27	<u>31</u>
JK4 For	5' TTT TAT TTC CAA CTT TGT CCC CGA 3'	28	<u>32</u>
JK5 For	5' TTT CAG CTC CAG CTT GGT CCC AGC 3'	29	<u>33</u>

C. Reamplification primers containing restriction sites

Mouse VH Sfi back primers

VH1 Sfi	5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTT CAG GAG TCA GG 3'	30	<u>34</u>
VH2 Sfi	5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAT GTG CAG CTT CAG GAG TCR GG 3'	31	<u>35</u>
VH3 Sfi	5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG AAG SAG TCA GG 3'	32	<u>36</u>
VH4/6 Sfi	5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTY CAG CTG CAR CAR TCT GG 3'	33	<u>37</u>
VH5/9 Sfi	5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTY CAR CTG CAG CAG YCT GG 3'	34	<u>38</u>
VH7 Sfi	5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAR GTG AAG CTG GTG GAR TCT GG 3'	35	<u>39</u>
VH8 Sfi	5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTT CAG CTT CAG CAG TCT GG 3'	36	<u>40</u>
VH10 Sfi	5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAA GTG CAG CTG KTG GAG WCT GG 3'	37	<u>41</u>
VH11 Sfi	5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG ATC CAG TTG CTG CAG TCT GG 3'	38	<u>42</u>

3 Mouse JK Not forward primers

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JK1 Not	5' GAG TCA TTC TCG ACT TGC GGC CGC TTT GAT TTC CAG CTT GGT GCC TCC 3'	39	<u>43</u>
JK2 Not	5' GAG TCA TTC TCG ACT TGC GGC CGC TTT TAT TTC CAG CTT GGT CCC CCC 3'	40	<u>44</u>
JK3 Not	5' GAG TCA TTC TCG ACT TGC GGC CGC TTT TAT TTC CAG TCT GGT CCC ATC 3'	41	<u>45</u>
JK4 Not	5' GAG TCA TTC TCG ACT TGC GGC CGC TTT TAT TTC CAA CTT TGT CCC CGA 3'	42	<u>46</u>
JK5 Not	5' GAG TCA TTC TCG ACT TGC GGC CGC TTT CAG CTC CAG CTT GGT CCC AGC 3'	43	<u>47</u>

R = A/G, Y = C/T, S = G/C, K = G/T, W = A/T, M = A/C, V = C/G/A, B = G/C/T, and H = C/A/T.

At pages 47-48, amend paragraph 0166 as follows:

[0001] scFv gene repertoires were assembled from purified V_H and V_K gene repertoires and linker DNA by using splicing by overlap extension. Linker DNA encoded the peptide sequence (G₄S₃, SEQ ID NO:45_278) Huston, *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883) and was complementary to the 3' ends of the rearranged V_H genes and the 5' ends of the rearranged V_K genes. The V_H and V_K DNAs (1.5 µg of each) were combined with 500 ng of linker DNA (Recombinant Phage Antibody System; Pharmacia Biotech) in a 25 µl PCR mixture containing 250 µm (each) deoxynucleoside triphosphate, 1.5 mM MgCl₂, 10 µg of bovine serum albumin/ml, and 1 µl (5 U) of Taq DNA polymerase (Promega) in the buffer supplied by the manufacturer, and the mixture was cycled 10 times (at 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min) to join the fragments. Flanking oligonucleotide primers (RS, provided in the Recombinant Phage Antibody System kit, for library I and an equimolar mixture of V_HSfi and JKNot primers [Table 1] for library 2) were added, and the reaction mixture was cycled for 33 cycles (at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min) to append restriction sites.

At page 57, replace Table 4 with the accompanying replacement Table 4 (4 pages).

At pages 63-64 amend paragraph 0198 as follows:

[0002] V_H genes of C25, S25, and 3D12 single-chain fragment variable (scFv) were amplified using PCR from the respective phagemid DNA with the primer pairs GTC TCC TGA GCT AGC TGA GGA GAC GGT GAC CGT GGT (SEQ ID NO:44_96) and either GTA CCA ACG CGT GTC TTG TCC CAG GTC CAG CTG CAG GAG TCT (C25, SEQ ID NO:45_97), GTA CCA ACG CGT GTC TTG TCC CAG GTG AAG CTG CAG CAG TCA (S25, SEQ ID NO:46_98), or GTA CCA ACG CGT GTC TTG TCC CAG GTG CAG CTG GTG CAG TCT (3D12, SEQ ID NO:47_99). DNA was digested with Mlu1 and NheI, ligated into N5KG1Val- Lark (gift of Mitch Reff, IDEC Pharmaceuticals, San Diego) and clones containing the correct V_H identified by DNA sequencing. V_K genes of C25, S25, and 3D12 scFv were amplified from the respective phagemid DNA with the primer pairs TCA GTC GTT GCA TGT ACT CCA GGT GCA CGA TGT GAC ATC GAG CTC ACT CAG TCT (SEQ ID NO:48_100) and CTG GAA ATC AAA CGT ACG TTT TAT TTC CAG CTT GGT (C25, SEQ ID NO:49_101), TCA GTC GTT GCA TGT ACT CCA GGT GCA CGA TGT GAC ATC GAG CTC ACT CAG TCT (SEQ ID NO:50_102) and CTG GAA

ATC AAA CGT ACG TTT GAT TTC CAG CTT GGT (S25, SEQ ID NO:54103), or TCA GTC GTT GCA TGT ACT CCA GGT GCA CGA TGT GAC ATC GTG ATG ACC CAG TCT (SEQ ID NO:52104) and CTG GAA ATC AAA CGT ACG TTT TAT CTC CAG CTT GGT (3D12, SEQ ID NO:53105), cloned into pCR-TOPO (Invitrogen) and clones containing the correct V_H identified by DNA sequencing. V_{genes} were excised from pCR-TOPO with *Dra*III and *Bsi*WI and ligated into *Dra*III- and *Bsi*WI-digested N5KG1Val-Lark DNA containing the appropriate V_H gene. Clones containing the correct V_H and V_K gene were identified by DNA sequencing, and vector DNA was used to transfect CHO DG44 cells by electroporation. Stable cell lines were established by selection in G418 and expanded into 1L spinner flasks. Supernatant containing IgG was collected, concentrated by ultrafiltration, and purified on Protein G (Pharmacia).

At pages 79-81, please amend Table 9 as follows:

Table 9. CDR 3-sequences and affinities for human scFv antibodies isolated from immune and non-immune libraries, selected on BoNT/A and BoNT/A H_C.^a

Non-immune library Heavy Chain				V _H CDR3
Clone	Family	Segment	Diff from Genome	V _H CDR3
2A9 ^b	V _H 3	DP54	5	GRGVN (SEQ ID NO: <u>54</u> <u>106</u>)
2B1 ^b	V _H 3	DP46	0	NGDPEAFDY (SEQ ID NO: <u>55</u> <u>-107</u>)
2H6 ^b	V _H 3	DP47	6	ALQSDSPYFD (SEQ ID NO: <u>56</u> <u>-108</u>)
3C2 ^b	V _H 3	DP46	2	DLAIFAGNDY (SEQ ID NO: <u>57</u> <u>-109</u>)
2B6 ^b	V _H 3	DP47	3	VGVDRWYPADY (SEQ ID NO: <u>58</u> <u>-110</u>)
3F6 ^c	V _H 3	DP47	2	DLLDGSGAYFDY (SEQ ID NO: <u>59</u> <u>-111</u>)
2A2 ^b	V _H 3	DP46	0	DLDYGGNAGYFDL (SEQ ID NO: <u>60</u> <u>-112</u>)
2B10 ^b	V _H 3	DP46	0	DLDYGGNAGYFDL (SEQ ID NO: <u>61</u> <u>-113</u>)
2E6 ^b	V _H 3	DP46	0	DYTANYYYYGMDV (SEQ ID NO: <u>62</u> <u>-114</u>)

3D1b	V _H 3	DP47	7	DLGYGSGTSSYYLDY (SEQ ID NO: <u>63-115</u>)
Non-immune library Light Chain				V _L CDR3
2A9 ^b	V _k 1	L12A	6	QQANSFPRT (SEQ ID NO: <u>64-116</u>)
2B1 ^b	V _k 1	L1	11	LQDYNGWT (SEQ ID NO: <u>65-117</u>)
2H6 ^b	V _λ 3	DPL16	7	NSRDSSGNHV (SEQ ID NO: <u>66-118</u>)
3C2 ^b	V _λ 3	DPL16	9	KSRDSRGNHLAL (SEQ ID NO: <u>67-119</u>)
2B6 ^b	V _k 1	L12A	5	QQYHTISRT (SEQ ID NO: <u>68-120</u>)
3F6 ^c	V _λ 3	DPL16	3	NSRDSSGNHV (SEQ ID NO: <u>69-121</u>)
2A2 ^b	V _λ 3	DPL16	10	HSRDSSVTNLD (SEQ ID NO: <u>70-122</u>)
2B10 ^b	V _λ 3	DPL16	4	NSRDSSGNHQV (SEQ ID NO: <u>71-123</u>)
2E6 ^b	V _λ 2	DPL12	14	NSRDSSGVV (SEQ ID NO: <u>72-124</u>)
3D1 ^b	V _λ 3	DPL16	5	NSRDSSGNHV (SEQ ID NO: <u>73-125</u>)
Immune Library Heavy Chain				
Clone	Family	Segment	Diff from Genome	V _H CDR3
3B8 ^c	V _H 1	V1-2	10	LATYYYFGLDV (SEQ ID NO: <u>74-126</u>)
3F10 ^c	V _H 1	V1-2	10	LATYYYFGLDV (SEQ ID NO: <u>75-127</u>)
2B11 ^c	V _H 1	DP10	11	GPWELVGYFDS (SEQ ID NO: <u>76-128</u>)
3A6c	V _H 3	DP50	18	EPDWLLWGDRGALDV (SEQ ID NO: <u>77-129</u>)
3D12 ^c	V _H 3	DP50	13	EPDWLLWGDRGALDV (SEQ ID NO: <u>78-130</u>)
2A1 ^b	V _H 3	DP50	14	EPDWLLWGDRGALDV (SEQ ID NO: <u>79-131</u>)
Immune Library Light Chain				
Clone	Family	Segment	Diff from	V _L CDR3

			Genome	
3B8 ^c	V κ 1	DPK7	12	QQYNSYVYT (SEQ ID NO: <u>80_132</u>)
3F10 ^c	V κ 1	DPK8	10	QQLNSYPLT (SEQ ID NO: <u>81_133</u>)
2B11 ^c	V κ 1	L12	11	QQLISYPLT (SEQ ID NO: <u>82_134</u>)
3A6 ^c	V κ 1	L12	8	QHYNTYPYT (SEQ ID NO: <u>83_135</u>)
3D12 ^c	V κ 1	L12	10	QHYNTYPYT (SEQ ID NO: <u>84_136</u>)
2A1 ^b	V κ 1	L12	4	QHYNTYPYT (SEQ ID NO: <u>85_137</u>)

^a Human germline VH , V κ and V λ segments have been assigned as detailed in the V-BASE database (MRC Centre for Protein Engineering, Cambridge, UK). Listed clones, with identical VH or VL CDR 3 regions, showed different CDR 1, CDR 2 and framework regions, as indicated by their differences from the germline genes; accession can be made through GenBank with nos. AF090405–AF090420.

^b Library selected on BoNT/A.

^c Library selected on BoNT/A HC.

At pages 85-88, amend Table 11 as follows:

[0241] Table 11 amino acid sequences for affinity matured and/or modified antibodies.

Heavy Chains				
Clone	Framework 1	CDR1	Framework 2	CDR2
huC25	QVQLQESGGGLVQPQGGSLRLSC AASGFTFS (SEQ ID NO: <u>86138</u>)	DYYMY (SEQ ID NO: <u>87139</u>)	WVRQAPGKGLEW VA (SEQ ID NO: <u>88140</u>)	TISDGGSYTYYPD SVKG (SEQ ID NO: <u>89141</u>)
Ar1	QVQLQESGGGLVQPQGGSLRLSC AASGFTFS (SEQ ID NO: <u>90 142</u>)	DYYMY (SEQ ID NO: <u>91 143</u>)	WVRQAPGKGLEW VA (SEQ ID NO: <u>92144</u>)	TISDGGSYTYYPD SVKG (SEQ ID NO: <u>93145</u>)
Ar2	QVQLQESGGGLVQPQGGSLRLSC AASGFTFS (SEQ ID NO: <u>94146</u>)	DHYMY (SEQ ID NO: <u>95147</u>)	WVRQAPGKGLEW VA (SEQ ID NO: <u>96148</u>)	TISDGGSYTYYPD SVKG (SEQ ID NO: <u>97149</u>)
WR1(V)	QVQLQESGGGLVQPQGGSLRLSC AASGFTSS (SEQ ID NO: <u>98150</u>)	DHYMY (SEQ ID NO: <u>99151</u>)	WVRQAPGKGLEW VA (SEQ ID NO: <u>100152</u>)	TISDGGSYTYYPD SVKG (SEQ ID NO: <u>101153</u>)
WR1(T)	QVQLQESGGGLVQPQGGSLRLSC AASGFTSS (SEQ ID NO: <u>102154</u>)	DHYMY (SEQ ID NO: <u>103155</u>)	WVRQAPGKGLEW VA (SEQ ID NO: <u>104156</u>)	TISDGGSYTYYPD SVKG (SEQ ID NO: <u>105157</u>)
3D12	QVQLVQSGGGVVHPGRSLKLSC AGSGFTFS (SEQ ID NO: <u>106158</u>)	DYDMH (SEQ ID NO: <u>107159</u>)	WVRQAPGKGLEW VA (SEQ ID NO: <u>108160</u>)	VMWFDGTEKYSAE SVKG (SEQ ID NO: <u>109161</u>)
3-1	QVQLVQSGGGVVHPGRSLKLSC AGSGFTFS (SEQ ID NO: <u>110162</u>)	DYDMH (SEQ ID NO: <u>111163</u>)	WVRQAPGKGLEW VA (SEQ ID NO: <u>112164</u>)	VMWFDGTEKYSAE SVKG (SEQ ID NO: <u>113165</u>)

3-8	QVQLVQSGGGVVHPGRSLKLSC AGSGFTFS (SEQ ID NO: <u>114166</u>)	DYDMH (SEQ ID NO: <u>115167</u>)	WVRQAPGKGLEW VA (SEQ ID NO: <u>116168</u>)	VIWFDGTEKYS SAE SVKG (SEQ ID NO: <u>117169</u>)
3-10	QVQLVQSGGGVVHPGRSLKLSC AGSGFTFS (SEQ ID NO: <u>118170</u>)	DYDMH (SEQ ID NO: <u>119171</u>)	WVRQAPGKGFEW VA (SEQ ID NO: <u>120172</u>)	VMWFDGTEKYS SAE SVKG (SEQ ID NO: <u>121173</u>)
ING1	QVQLQQSGGGLVQPGGSLRLSC AASGFTFS (SEQ ID NO: <u>122174</u>)	NYAMT (SEQ ID NO: <u>123175</u>)	WVRQAPGKGLEW VS (SEQ ID NO: <u>124176</u>)	SISVGGSDTYYAD SVKG (SEQ ID NO: <u>125177</u>)
Heavy Chains cont'd				
	Framework 3	CDR3	Framework 4	
huC25	RFTISRDNSKNTLYLQMNSLRA EDTAIYYCSR (SEQ ID NO: <u>126178</u>)	YRYDDAMDY (S EQ ID NO: <u>127179</u>)	WGQGTLTVSS (SEQ ID NO: <u>128180</u>)	
Ar1	RFTISRDNSKNTLYLQMNSLRA EDTAIYYCSR (SEQ ID NO: <u>129181</u>)	YRYDDAMDY (S EQ ID NO: <u>130182</u>)	WGQGTLTVSS (SEQ ID NO: <u>131183</u>)	
Ar2	RFTTSRDNSKNTLYLQMNSLRA EDTAIYYCSR (SEQ ID NO: <u>132184</u>)	YRYDDAMDY (S EQ ID NO: <u>133185</u>)	WGQGTLTVSS (SEQ ID NO: <u>134186</u>)	
WR1(V)	RFTVSRDNSKNTLYLQMNSLRA EDTAIYYCSR (SEQ ID NO: <u>135187</u>)	YRYDDAMDY (S EQ ID NO: <u>136188</u>)	WGQGTLTVSS (SEQ ID NO: <u>137189</u>)	
WR1(T)	RFTTSRDNSKNTLYLQMNSLRA EDTAIYYCSR (SEQ ID NO: <u>138190</u>)	YRYDDAMDY (S EQ ID NO: <u>139191</u>)	WGQGTLTVSS (SEQ ID NO: <u>140192</u>)	
3D12	RFTISRDNSKNTLFLQMNSLRA DDTAVYYCAR (SEQ ID NO: <u>141193</u>)	EPDWLLWGDRG ALDV (SEQ ID NO: <u>142194</u>)	WGQGTTTVSS (SEQ ID NO: <u>143195</u>)	
3-1	RFTISRDNSKNTLFLQMNSLRA DDTAVYYCAR (SEQ ID NO: <u>144196</u>)	EPDWLLWGDRG ALDV (SEQ ID NO: <u>145197</u>)	WGQGTTTVSS (SEQ ID NO: <u>146198</u>)	
3-8	RFTISRDNSKNTLFLQMNSLRA DDTAVYYCAR (SEQ ID NO: <u>147199</u>)	EPDWLLWGDRG ALDV (SEQ ID NO: <u>148200</u>)	WGQGTTTVSS (SEQ ID NO: <u>149201</u>)	
3-10	RFTISRDNSKNTLFLQMNSLRA DDTAVYYCAR (SEQ ID NO: <u>150202</u>)	EPDRLLWGDRG ALDV (SEQ ID NO: <u>151203</u>)	WGQGTTTVSS (SEQ ID NO: <u>152204</u>)	
ING1	RFTVSRDNSKNTLLLQMNSLRA EDTAVYYCAK (SEQ ID NO: <u>153205</u>)	VRTKYCSSLSC FAGFDS (SEQ ID NO: <u>154206</u>)	WGQGTLTVSS (SEQ ID NO: <u>155207</u>)	
Light Chains				

Clone	Framework 1	CDR1	Framework 2	CDR2
huC25	EIVLTQSPATLSLSPGERATIS C (SEQ ID NO:156208)	RASESVDSYGH SFMQ (SEQ ID NO:157209)	WYQQKPGQAPRL LIY (SEQ ID NO:158210)	RASNLEP (SEQ ID NO:159211)
Ar1	EIVLTQSPATLSLSPGERATIS C (SEQ ID NO:160212)	RASESVDSYGH SFMQ (SEQ ID NO:161213)	WYQQKPGQAPRL LIY (SEQ ID NO:162214)	RASNLEP (SEQ ID NO:163215)
Ar2	EIVLTQSPATLSLSPGERATIS C (SEQ ID NO:164216)	RASESVDSYGH SFMQ (SEQ ID NO:165217)	WYQQKPGQAPRL LIY (SEQ ID NO:166218)	RASNLEP (SEQ ID NO:167219)
WR1(V)	EIVLTQSPATLSLSPGERATIS C (SEQ ID NO:168220)	RASESVDSYGH SFMQ (SEQ ID NO:169221)	WYQQKPGQAPRL LIY (SEQ ID NO:170222)	RASNLEP (SEQ ID NO:171223)
WR1(T)	EIVLTQSPATLSLSPGERATIS C (SEQ ID NO:172224)	RASESVDSYGH SFMQ (SEQ ID NO:173225)	WYQQKPGQAPRL LIY (SEQ ID NO:174226)	RASNLEP (SEQ ID NO:175227)
3D12	DIVMTQSPSTLSASVGDRVTIT C (SEQ ID NO:176228)	RASQSISSWLA (SEQ ID NO:177229)	WYQQKPGKAPKL LMY (SEQ ID NO:178230)	EASSLES (SEQ ID NO:179231)
3-1	DIVMTQSPSTLSASVGDRVTIT C (SEQ ID NO:180231)	WASQSISSRLA (SEQ ID NO:181233)	WYQQKPGKAPKL LMY (SEQ ID NO:182234)	EATSLGS (SEQ ID NO:183235)
3-8	DIVMTQSPSTLSASVGDRVTIT C (SEQ ID NO:184236)	RASQSISSWLA (SEQ ID NO:185237)	WYQQKPGKAPKL LMY (SEQ ID NO:186238)	GASSLGS (SEQ ID NO:187239)
3-10	DIVMTQSPSTLSASVGDRVTIT C (SEQ ID NO:188240)	RASQSISSWLA (SEQ ID NO:189241)	WYQQKPGKAPKL LMY (SEQ ID NO:190242)	EASSLGR (SEQ ID NO:191243)
ING1	DIVMTQSPSSLASVGDRVTIT C (SEQ ID NO:192244)	RASQSISSYLN (SEQ ID NO:193245)	WYQQKPGKAPKL LIY (SEQ ID NO:194246)	AASSLQS (SEQ ID NO:195247)
Light Chains cont'd.				
Clone	Framework 3	CDR3	Framework 4	
huC25	GIPARFSGSGSGTDFTLTISSL EPEDFAVYYC (SEQ ID NO:196248)	QQSNEDPFT (SEQ ID NO:197249)	FGQGTKVEIKR (SEQ ID NO:198250)	
Ar1	GIPARFSGSGSGTDFTLTISSL EPEDFAVYYC (SEQ ID NO:199251)	QQGNEVPFT (SEQ ID NO:200252)	FGQGTKVEIKR (SEQ ID NO:201253)	
Ar2	GIPARFSGSGSGTDFTLTISSL EPEDFAVYYC (SEQ ID	QQGNEVPFT (SEQ ID	FGQGTKVEIKR (SEQ ID	

	NO: <u>202254</u>)	NO: <u>203255</u>)	NO: <u>204256</u>)	
WR1(V)	GIPARFSGSGSGTDFTLTISSL EPEDFAVYYC (SEQ ID NO: <u>205257</u>)	QQGNEVPFT (SEQ ID NO: <u>206258</u>)	FGQGTKVEIKR (SEQ ID NO: <u>207259</u>)	
WR1(T)	GIPARFSGSGSGTDFTLTISSL EPEDFAVYYC (SEQ ID NO: <u>208260</u>)	QQGNEVPFT (SEQ ID NO: <u>209261</u>)	FGQGTKVEIKR (SEQ ID NO: <u>210262</u>)	
3D12	GVPSRFSGSGSGTEFTLTSSL QPDDFAAYYC (SEQ ID NO: <u>211263</u>)	QHYNTYPYT (SEQ ID NO: <u>212264</u>)	FGQGTKLEIKR (SEQ ID NO: <u>213265</u>)	
3-1	GVPSRFSGSGSGTEFTLTSSL QPDDFAAYYC (SEQ ID NO: <u>214266</u>)	QHYDTYPYT (SEQ ID NO: <u>215267</u>)	FGQGTKLEIKR (SEQ ID NO: <u>216268</u>)	
3-8	GVPSRFSGSGSGTEFTLTSSL HPDDFAAYYC (SEQ ID NO: <u>217269</u>)	QHYNTYPYT (S EQ ID NO: <u>218270</u>)	FGQGTKLEIKR (SEQ ID NO: <u>219271</u>)	
3-10	GVPSRFSGSGSGTEFTLTSSL QPDDFAAYYC (SEQ ID NO: <u>220272</u>)	QHYSTYPYT (S EQ ID NO: <u>221273</u>)	FGQGTKLEIKR (SEQ ID NO: <u>222274</u>)	
ING1	GVPSRFSGSGSGTDFTLTISSL QPEDFATYYC (SEQ ID NO: <u>223275</u>)	QQSYSTPRTT (S EQ ID NO: <u>224276</u>)	FGGGTKVDIKR (SEQ ID NO: <u>225277</u>)	

*Sequence for complete heavy chain is heavy chain framework 1+ CDR1 + framework 2 + CDR2 + framework 3 + CDR3 + framework 4.

Sequence for complete light chain is light chain framework 1+ CDR1 + framework 2 + CDR2 + framework 3 + CDR3 + framework 4.

Table 4. Deduced protein sequences of VH and VL of BoNT/A Hc binding scFv classified by epitope recognized.

V _H Region	Epitope 1	Sequence ^b			
		Framework 1		Framework 2	
Clo ne	Lib ^a	Framework 3	CDR 1 CDR3	Framework 4	CDR 2 Seq ID No
C15 1	QVKLQQSGAELVRPGAVKLSCKTSGY SFT	SYWMN GIYYDGGNYYAMDY	WVKQGPQGGLEWIG WGQGTTVTVASS	MIHPNSNEIRFNQKFED 48	
C9 1	QVKLQQSGAELVRPGAVKLSCKTSGY SFT	SYWMN GIYYDGGNTTAMDY	WVKQGPQGGLEWIG WGQGTTVTVASS	MIHPNSNEIRFNQKFEN 49	
1D5 2	eVKLveSGAELVRPGAVNLCKASGY SFT	SYWMN GIYYDGYDeGYYT1DY	WVKQRPQGGLEWIG WGQGTT1TVSS	MIHPNSNEtrLNQKFKD 50	
C1 1	QVKLQQSGAELVRPGAVKLSCKA SGY SFT	SYWMN Glygygf	WVKQRPQGGLEWIG WGQGTTVTVSS	MIHPNSNSdtRFNQKFED 51	
S25 1	QVKLQQSGAELVRPGAVKLSCKASGY S1T	SYWMN Glyngf	WVKQRPQGGLEWIG WGQGTTVTVSS	MIHPSSdSdtRFNQKFED 52	
1B6 2	QVqlQQSGAELVRPGAVKISCKASGY F1	DYAMH Rgkg	WVKQSPaksLEWIG WGQGTTVTVSS	vissyygdtddyNQiFkg 53	
1C9 2	QVqlLkQQSGAELVRPGAVSvkisCKASGY Fi	DYAVH Rgkg	WVKQshaksLEWIG WGQGTSVTVSS	vistyyggdadynPpkFkg 54	
1E8 2	eVqlQesGpgLVkPsqS1sltCtvGYS1T	dyawn Gyd	WirkQPGkLKLEWmG WGQGTSVTVSS	y1s ysgstgynpslks 55	
1G7 2	eVqlQesGpgLVkPsqS1sltCtvGYS1T	dyawy Gyd	WirkQPGkLKLEWmG WGQGTSVTVSS	y1s ysgstgynpslks 56	
Epitope 2					
1A1 2	EVKLVEGGGLVQPGGSRKLSCATSGFTFS	DYMS	WIRQSPDKRLEWVA WGAGTTVTVSS	TISDGTYTYYPPDSVKG 57	
1F1 2	EVKLVESGGGLVQPGGSLK1SCAA SGFTFS	nyGMS	WvRQtPDKRLEWVA WGAGTTVTVSS	m1SsGsyNySDSVKG 58	
C39 1	qVqlqeSGGGsvkPGGS1K1SCAA SGFTFS	DYMS	WvRQtPekRLEWVA WGAGTTVTVSS	TISDGGSYTYYPPDSVKG 59	
C25 1	RFT1SRDNAKNAKNLYLQMSLKS aSGFTFS	DYMY Yrydegl	WvRQtPekRLEWVA WGAGTTVTVSS	TISDGGSYTYYPPDSVKG 60	
2G5 2	RFT1SRDNAKNAKNLYLQMSLKS aSGFTFS	SYAMS nlpvdhv	WvRQtPekRLEWVA WGqGTTVTVSS	TISDGTYTYYtDnVKG 61	
3C3 2	EVKLKEGGGLVkPGGS1K1SCAA SGFTFS	SYAMS	WvRQtPekRLEWVA	TISDGTYTYYtDnVKG 61	

3F4	2	RFTISRDNAKhnLYLQMShLKS EDTAMYYCaR	nlpdydhv	Dy	WGGGTsvTVSS	62
		RFTISRDNAKhnLYLQMShLKS EDTAMYYCaR	syAMS	WvrQtPehrLEWA	TISDGtfTYYtDnVKG	
3H4	2	EVKLVESGGGLVkpGGS1kLSCAaSGFTFS	nlpdydhv	Dy	WGQGtsVTvSS	63
		EVKLVESGGGLVkpGGS1kLSCAaSGFTFS	syAMS	WvrQtPehrLEWA	TISDGtfTYYtDnVKG	
Epitope 3			nlpdydhv	Dy	WGQGtsVTvSS	64
1B3	2	EVQLQESGGGVVQPGRSRLRLSCAASGFTFS	SYAMH	WVRQAPGKGGLEWA	VISYDGSNKYYADSVKG	
		RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DWSEGYYYYG	MDV	WGQGTTVIVSS	
1C6	2	qIQLIqSGGGVVQPGRSRLRLSCAASGFTFS	SYAMH	WVRQAPGKGGLEWA	VISYDGSNKYYADSVKG	
		RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DWSEGYYYYG	MDV	WGQGTTVIVSS	
2B6	2	vk1vesgpG1Vkpssoslsllctvtgysisits	dyawn	WirofPgnklewmg	yInyDGSNnYnp	S1kn
		RisitRDTSKRNqfflk1nsVtsedtAtYYCAR	AgdgyYvd	wytfdv	WTkQrpGqglewig	67
1G5	2	qVQLQqSGaelVQPGaSvkmsCKASGYTFT	dywtt	WVtkQrpGqglewig	dIypgsgstnynekfks	
		kat1tvDtsstsTaYmQ1ssltsedsAVYYCAR	Elgd	aMDy	WGQGtsVTvSS	68
1H6	2	EVQLQQSGaelVQPGaSvkmsCKASGYTFT	dywtt	WVtkQrpGqglewig	dIypdsgstnynekfks	
		kat1tvDtsstsTaYmQ1ssltsedsAVYYCAR	Elgd	aMDy	WGQGtsVTvSS	69
Epitope 4						
1F3	2	EVQLQQSGAELVKGASVYKLSCKASGYTFT	SFWMH	WVKQRPGRGLEWIG	RLDPNGETKYNKEFKKS	
		KATLTVDKPSSTAYMELSSLTSedsAVYYCAR	EAYGWN	FDV	WTGTTVIVSS	70
2E8	2	EVQLQQSGAELVKGASVYKLSCKASGYTFT	SFWMH	WVKQRPGRGLEWIG	RLDPNGETKYNKEFKKS	
		KATLTVDKPSSTAYMELSSLTSedsAVYYCAR	EAYGWN	FDV	WTGTTVIVSS	71

V^r Region
Epitope 1

Clone	Lib	Framework 1	CDR 1	Framework 2	CDR 2
		Framework 3	CDR 3	Framework 4	Seq ID
C15	1	DIELTQSPAIMSASPGEKViMTC	SASS	WYQQKPGSSPRLLIY	DTSNLAS
		QVPVRFSGSGSGTYSLTISMEAEDSATYYC	' QQWISSYPFT	FGSGTKLEiKR	72
C9	1	DIELTQSPAIMSSSPGEKViITC	SASS	WfQQRPGtSPkpiWY	STSNLAS
		QVPVaRFSGSGSGTYSLTISMEAEDAATYYC	QQySgyp1T	FGaGtKLEiKR	73
1D5	2	DIELTQSPAIMMaASPGEKViITC	SASSs	WYQQKsetspkpiWY	9TSNLAS
		QVPVRFSGSGSGTYSLTISMEAEDAATYYC	QQWgSgyp1T	FGggGtKLEiKR	74
C1	1	DIELTQSPAIMSASPGEKViMTC	SASS	WYQQKPGSSPRLLIY	DTSNLAS
		QVPVRFSGSGSGTYSLTISMEAEDAATYYC	QQWISSYP1T	FGaGtKLEiKR	75
S25	1	DIELTQSPAIMMaASPGEKViITC	SvSSs	WYQQKSGtSPkpiWY	9TSNLAS
		QVPVRFSGSGSGTYSLTISMEAEDAATYYC	QQWISSYP1T	FGaGtKLEiKR	76
1B6	2	DIELTQSPASlavS1Gqrailsc	raYesvdsygnSFmh	WYQQKPGQppkliY	raSNLeS

1C9	2	QIPARFSGSGSRTdftLTInpveAddATYYC	QQsnedPPT	FGaGTTKLEIKR	77
1E8	2	QIPARFSGSGSRTdftLTInpveAddATYYC	raYesvdsygnSFmh	WYQQKPGqpPKLILY	raSNLeS
1E8	2	DIELTQSPAIMSASPGEKVIIMTC	SASS	FGaGTTKLEIKR	78
1G7	2	QIPARFSGSGSRTSYSLTISMEAEDAATYYC	SASS	WYQQKSGtSPkrWIY	DTSKLAS
		DIELTQSPAIMSASPGEKVIIMTC	SASS	FGaGTTKLEIKR	79
		QIPARFSGSGSRTSYSLTISMEAEDAATYYC	SASS	WYQQKSGtSPkrWIY	DTSKLAS
			QQwISSnPlT	FGaGTTKLEIKR	80
1A1	2	DIELTQSPASLAVSLGQRATISC	RASESDYGNsFMG	WYQQKPGQPpPKLILY	LASNLES
		GVPARFSGSGSRTDFLTIDPVEADDAATYYC	QWmSSYPFT	FGSGTGTKEIKR	81
1F1	2	DIELTQSPtSLAVSLGQRATISC	RASESDYGNsFMH	WYQQKPGQPpPKLILY	LASNLES
		GVPARFSGSGSRTDFLTIDPVEADDAATYYC	QQySgyPlT	FGSGTGTKEIKR	82
C39	1	DIELTQSPASLAVSLGQRATISC	RASESDYGNsFMH	WYQQKPGQPpPKLILY	LASNLES
		GVPARFSGSGSRTDFLTIDPVEADDAATYYC	QWmSSYPFT	FGSGTGTKEIKR	83
C25	1	DIELTQSPASLAVSLGQRATISC	RASESDYGNsFMq	WYQQKPGQPpPKLILY	rASNLeP
		GIPARFSGSGSGtDfRLTInpveAddATYYC	QQwISSYpT	FGSGTGTKEIKR	84
2G5	2	DIELTQSPAlmsaSpGekvttC	SASS	svSYMG	WFQQKPGtSPkLWIY
		GVPARFSGSGSGtSYSLTIsrmEAeDAATYYC	QQsnedPPT	stSNLeS	85
3C3	2	DIELTQSPAlmsaSpGekvttC	RASESDYGNsFMq	WFQQKPGtSPkLWIY	stSNLeS
		GVPARFSGSGSGtSYSLTIsrmEAeDAATYYC	QQsnedPPT	FGSGdqaGnKS	86
3F4	2	DtELTQSPAlmsaSpGekvttC	SASS	svSYMY	WFQQKPGssPrLILY
		GVPvRFSGSGSGtSYSLTIsrmEAeDAATYYC	QQwISSnPlT	FGSGTGTKEIKR	dtSNLeS
3H4	2	DIELTQSPAlmsaSpGekvttC	RASSs	vssSy1G	WYQQKPGssPrLILY
		GVPvRFSGSGSGtSYSLTIsrmEAeDAATYYC	QQwISSnPlT	FGSGTGTKEIKR	dtSNLeS
					88
1B3	2	DSELTQSPTTMAASPGEKTTTC	SASSs	ISSNylH	RTSNLeS
		GVPARFSGSGSGtSYSLTIGTMEAEDVATYYC	QGQSSIPRT	FGGGTGTKEIKR	89
1C6	2	DIELTQSPAs1AVSLGrrartSC	raSeSveyygts1mq	WYQQKPGqpPKLILY	aasNvEs
		GVPARFSGSGSGtDFSLnIhpvEe DiAmYFC	QOsrlkvPwt	FGGGTGTKEIKR	90
2B6	2	yIELTQSPAs1AVSLGrrartSC	raSeSvdsygnSmH	WYQQKPGqpPKLILY	laSNLeS
		GVPARFSGSGSGtDftLTIdpveAddAtYYC	QOrnedPyT	FGGGTGTKEIKS	91
1G5	2	DIELTQSPAs1AVSLGrrartSC	raSeSveyygts1mq	WYQQKPGqpPKLILY	aasNvEs
		GaPARFSGSGSGtDfSLnIhpvEedDiAmYfc	QOrirkvPyT	FGGGTGTKEIKR	92
1H6	2	DIELTQSPAlmsASPGEKVTTTC	SvSSs	WYQQKSGtSPkLWIY	9TSNLAS
		GVPvRFSGSGSGtSYSLTISMEAEDAATYYC	QQwISSnLH	FGaGTTKvElYR	93
1F3	2	DIELTQSPASMSASPGEKVMTc	RATss	VSSSYLH	SASNLeS
		GVPvRFSGSGSGtSYSLTISMEAEDAATYYC	QQYIGYPt	FGGGTGTKEIKR	94

2E8	2	DIELTQSPTMaaASPGEKiiTC GVPaRFSGSGSGTTSYSLTIGaveAEDVATYYC	sASSS QQgssiPYT	igSNYLH FGGGTRKLEIKR	WYQQKppGESPKLIIY FGGGTRKLEIKR	ktSNLAS 95
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^a Lib, library.

^b Full-length sequences were not determined for clones C12, C13, C2, and S44 (all bind epitope 1). Accession can be made through GenBank with

nos: AF003702 to AF003725.